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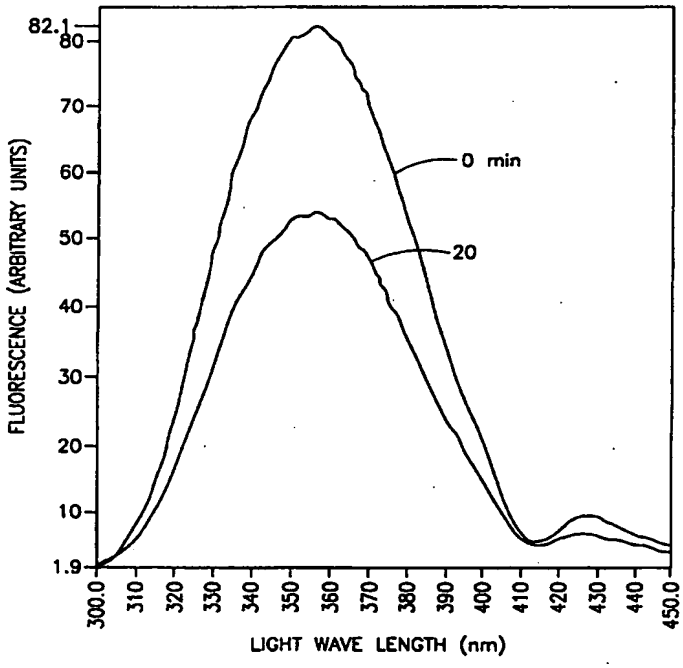
(57) Abstract

The present invention provides a method and system for destruction of biological tissues by various types of irradiation such as ultrasound or light using a helper agent or combination of agents, optionally together with an agent capable of being sensitized by the irradiation. The helper agent may release singlet oxygen, oxidizing compounds, free radicals or can be an agent capable of undergoing an exothermal reaction. The present invention further provides a method for terminating the destructive activity of various energy–sensitizable agents.



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PHOTODYNAMIC AND SONODYNAMIC THERAPY AND AGENTS AND SYSTEM FOR USE THEREFOR

FIELD OF THE INVENTION

The present invention concerns improvements in photodynamic therapy (PDT) and sonodynamic therapy (SDT).

BACKGROUND OF THE INVENTION

Photodynamic therapy (PDT) involves the use of photosensitizable compounds for the selective destruction of biological tissue, such as for the destruction of tumors, using a photosensitizable drug linked to tumor-localizing agent (for example an antibody), followed by exposure of the target region to light, usually light emitted from a laser (Kopecck, J., Targetable polymeric anticancer drugs- temporal control of drug activity: In Temporal control of drug delivery, eds.: W.J.M. Brushesky, R. Langer and F. Theeuwes, *Annals of the New York Academy of Sciences*, pp. 335-341 (1991)).

Photosensitizable compounds are molecules that are activated by light of a characteristic wavelength, usually from a laser, ultimately resulting in the formation of cytotoxic intermediates such as singlet oxygen or free radicals. The photosensitizable compound acts either at the cell surface, or is internalized, ultimately causing its destructive effect on the membrane at the cell surface or on cellular organella, respectively, both routes leading to cell death. In cancer treatment the tumor destruction is believed to proceed via one or both of the following two suggested mechanisms: the intravascular pathway, i.e. collapse of

blood vessels which hamper blood perfusion to the tumor and cause deprivation of oxygen and nutrients; and/or the parenchymal tumor pathways wherein destruction is caused due to direct necrotic effects on the tumor cells. One of the severe problems photodynamic therapy features is post-treatment sensitivity to sunlight, which requires to keep patients out of direct light for several weeks after photosensitizable compounds were administered (Orenstein *et al., Lasers Surg. Med.*, **10**:334-343 (1990)).

Sonodynamic therapy (SDT) is a new concept which relates to the synergistic effect of drugs and ultrasound in producing cytotoxic effect on tissue, in particular on tumors (Jeffers *et al., J. Acoust. Soc. Am.*, **97**:669-676 (1995)). The cytotoxicity of SDT can be enhanced by the presence of sonosensitizable compounds, i.e. agents which can emit singlet oxygen or free radicals in response to irradiation by ultrasound. Photosensitizable compounds, for example, porphyrin and porphyrinyl analogs, were found to be able to serve as sonosensitizable agents in cultures of tumor cells (Kessel *et al., J. Photochem. Photobiol.*, **B 28**:219-221 (1995)). The actual impact of the ultrasonic irradiation on the sonosensitizable compound is still obscure, but it has recently been reported that ultrasound irradiation of several compounds causes formation of free radicals such as $^{\bullet}CH_3$ and $^{\bullet}CH_2R$ radicals (Misik & Riesz, *Free Radical Biol. Med.*, **20**:129-128 (1996)). These radicals are formed either by reaction of the solutes with the ultrasound generating $^{\bullet}H$ and $^{\bullet}OH$ radicals of the water solution which can attack the sensitized compounds, or by direct pyrolysis of the weak bonds of the sensitizer to produce more free radicals in a chain reaction. It is believed that ultrasound destruction of cells *in vitro* is caused by the combined effect of ultrasound with the above mentioned compounds. Even though the underlying mechanisms have to be determined, they were at least partially attributed to the ultrasonic cavitation effects (Jeffers *et al., Supra* (1995)).

It would have been highly desirable to provide a method for selective destruction of biological tissue, by photodynamic, sonodynamic therapy

or other drug-energy combination, which would enhance the destructive or other desired effects of these therapies.

It would have further been desirable to provide a method which would minimize the post-treatment effect of delayed sensitivity to light, caused by photosensitizable compounds, which compounds may also be used as sonosensitized compounds.

SUMMARY OF THE INVENTION

The present invention concerns a method and agents for improving the destructive effects of various types of energy irradiation such as ultrasound irradiation alone, light and ultrasound irradiation together, or of other types of energy irradiation, on biological tissue.

Thus the present invention provides in accordance with a first aspect thereof a method for the destruction of biological tissue by an energy irradiation comprising exposing the biological tissue to energy irradiation in the presence of an agent capable of being sensitized by said irradiation, and at least one agent selected from the group consisting of:

(i) a compound capable of releasing singlet oxygen;

(ii) a compound capable of releasing oxidating agents; and

(iii) a compound capable of releasing free radicals.

The term *"energy irradiation"* refers to any type of irradiation which is biologically compatible, and includes light, ultrasound, microwave, radio, laser, etc. irradiations.

The term *"an agent capable of being sensitized by energy irradiation"* refers to specific agents which can be activated in the presence of energy, i.e. agents which change form in the presence of said irradiation so that they become active and may chemically react with biological tissue. Where the energy is light, this term refers to photosensitizable agents. Where the energy is ultrasound irradiation, this term is refers to sonosensitizable agents, etc.

In accordance with an aspect of the invention termed the *"sonodynamic therapy helper aspect"* destruction of the biological tissue is carried out in the presence of ultrasound and sonosensitizable agents, and a helper agent capable of contributing to the destructive affect of ultrasound for example, by releasing free radicals, singlet oxygens, oxidating agents and the like.

Thus in accordance with this aspect the present invention provides a method for the destruction of biological tissue by ultrasound irradiation comprising exposing the biological tissue to ultrasound irradiation in the presence of a sonosensitizable agent and at least one agent selected from the group consisting of:

(i) a compound capable of releasing singlet oxygen;

(ii) a compound capable of releasing oxidating agents; and

(iii) a compound capable of releasing free radicals.

The present invention further provides in accordance with a second aspect thereof a method for the destruction of tissue by energy irradiation comprising: exposing the biological tissue to irradiation in the presence of a composition of matter which upon exposure to said irradiation undergoes an exothermic reaction.

In accordance with the second aspect of the invention, termed *"sonodynamic therapy exothermic aspect"*, the present invention provides a method for the destruction of biological tissue by ultrasound irradiation utilizing new sonosensitizable agents capable of undergoing an exothermic reaction. The method for the destruction of the biological tissue comprises: exposing the biological tissue to ultrasound irradiation in the presence of a composition of matter which, upon exposure to said irradiation undergoes an exothermic reaction.

The destruction of biological tissue in the presence of a composition capable of undergoing an exothermic reaction, should be preferably carried out in the presence of a helper agent selected from the group consisting of:

(i) a compound capable of releasing singlet oxygen;

(ii) a compound capable of releasing oxidating agents; and

(iii) a compound capable of releasing free radicals.

Such a helper agent may contribute to the destructive effect as explained above. The sonodynamic therapy exothermic aspect of the invention, may be carried out in the presence of at least one other state of the art sonosensitizable compounds, which may contribute to the destructive effects of the exothermic agents.

Examples of sonosensitizable compounds are specified in Saad, A.H. and Hahn, G.M., *Cancer Res.*, **49**:5931-5934 (1989); Miyoshi, N. *et al., Radiat. Res.*, **143**:194-202 (1995); Misik V. and Riesz, P., *Free Radical Biol. Med.*, **20**:129-138 (1996); Kessel D. *et al., J. Photochem. Photobiol.*, **B28**:219-221, (1995); Jeffers, R.J. *et al., J. Acoust. Soc. Am.*, **97**:669-676 (1995); Tata, D. *et al., J. Acoust. Soc. Am.*, **93**:2348, (1993).

Examples of candidate sonosensitizable compounds are: gallium-porphyrin, DMSO (dimethylsulfoxide), DMF (dimethylformamide), mesoporphyrin, adramycin and mitomycin as well as other photosensitizable compounds which are known to act as sonosensitizable compounds.

The use of the helper agents (i) - (iii) above causes the destruction of the biological tissue by chemical interaction of the singlet oxygen, the oxidative or free radicals with biological macromolecules while the exothermic composition cause hyperthermic destruction of the biological tissue as will be explained hereinbelow. The simultaneous use of chemical and thermic destruction greatly enhance the effect of the sonodynamic therapy, either directly by initiating pyrolytic effect at the desired location or indirectly by increasing the local metabolism and the uptake of the sensitizer or increasing the reactivity of particular sensitizer for the desired chemical response.

The biological macromolecules attacked by the singlet oxygen, the oxidative or free radicals, are usually those with double bonds, or other sites rich

in electrons, such as many of the amino acids, unsaturized lipids, phospholipids, cholesterol, guanine and other molecules, which are all parts of membranes, proteins, nucleic acids and the like.

The first aspect of the invention may also be intended to enhance the destructive activity of photosensitizable compounds and is termed hereinafter the *"photodynamic therapy helper aspect"*. In accordance with this aspect the present invention concerns a method for the destruction of biological tissue by light, comprising exposing the biological tissue to light in the presence of a photosensitizable compound and at least one agent selected from the group consisting of:

(i) a compound capable of releasing singlet oxygen;

(ii) a compound capable of releasing oxidating agents; and

(iii) a compound capable of releasing free radicals.

As explained above, the above helper agents contribute to the destructive activity of the photosensitizable aspect.

In accordance with the second aspect of the invention termed: *"photodynamic therapy exothermic aspect"*, the present invention provides a method for the destruction of biological tissue by light comprising: exposing the biological tissue to light in the presence of a composition of matter which, upon exposure to said light, undergoes an exothermic reaction.

The composition of matter capable of undergoing an exothermic reaction should preferably be exposed to light in the presence of a helper agent, i.e. an agent selected from the group consisting of:

(i) a compound capable of releasing singlet oxygen;

(ii) a compound capable of releasing oxidatives; and

(iii) a compound capable of releasing free radicals.

The photodynamic therapy exothermic aspect of the invention may be carried out in the presence of at least one other state of the art photosensitizable agents.

Photosensitizable agents are well known in the art and are described, for example, in Gottfried, V. and Kimmel, S., *J. Photochem. Photobiol.*, **B8**:419-430 (1991); Gottfried, V., *et al., J. Photochem. Photobiol.*, **B30**:115-121 (1995); Kessel D. *et al., J. Photochem. Photobiol.*, **B28**:219-221 (1995); Margaron, P. *et al., Anticancer Res.*, **16**:613-620 (1996); Orenstein, A. *et al., Lasers Surg. Med.*, **10**:334-343, (1990).

Examples of such agents are: aromatic compounds including porphyrins, hematoporphyrins, chlorines, purpurins, phthalocyanines, photofrin, rhodamines, acridine derivated, rhodamines and the like.

The method according to the photodynamic therapy exothermic aspect of the invention enhances the cytotoxic effect of photosensitizable compounds by providing substrates for those compounds thus increasing the release of destructive agents such as free radicals, singlet oxygen and oxidatives (by utilizing helper agents (i) - (iii)). The composition of matter capable of undergoing an exothermic reaction in the presence of light, actually serve as novel sonosensitizable agents.

The term *"destruction of biological tissue"* refers to cytotoxic effect of ultrasound energy irradiation such as, light, microwave or other types of energy source, together with the appropriate compounds at the desired site leading to massive cell death at said site.

The term *"light"* refers to any type of light, preferably a monochrome wave of a single length which is chosen in accordance with the specific photosensitizable compound used. More preferably, higher wave length light should be used, such as red or infrared light, due to its higher penetration of biological tissue, in addition to its activation ability.

The term *"ultrasound"* refers to a mechanical wave with frequency above the audible range that propagates by motion of particles within the medium. The motion causes compression and refractions of the particles so that a pressure wave travels along with mechanical disturbance.

The helper agents of (i) - (iii) of the above aspects of the invention enhance the destruction activity caused by various types of energy irradiation, optionally together with agents which are sensitized by said energy irradiation.

Examples of compounds capable of releasing singlet oxygen are, for example O_2, provided by bubbling oxygen into the medium in which the biological compound is present, by using solutions wherein oxygen is a major and unstable component such as H_2O_2 or by using substances that can release oxygen such as kalium permanganat and the like.

Compounds capable of releasing oxidatives are chloride, bromide, fluoride, iodine, alkali metal elements (Na, K, Rb, Cs) and alkali earth metals (Be, Mg, Ca, Sr), oxygen and sulfur.

In general, oxidatives are molecules or single atoms which lack electrons or have a tendency to lose electrons in an aqueous solution. Information concerning oxidatives can be found in Dickerson, R.E., Gray, H.B. and Haight, G.P., Chemical principles, The Benjamin Cummings Publishing Company, California, pp, 358-398, (1979).

Compounds which are capable of generating free radicals such as $^{\bullet}CH_3$ and $^{\bullet}CH_2R$ radicals, are, for example, N,N-dimethylbromide (DMF), N-Me formamide (NMF) and dimethyl sulfoxide (DMSO). Ultrasound causes lysis and formation of radicals produced from the water itself. The radical produced from water may attack the above compounds (such as DMSO) to produce more free radicals. Alternatively, the ultrasound may attack directly the above compounds to produce free radicals.

The *"exothermic compositions"* are agents capable of initiating a strong exothermic reaction after being activated by ultrasound or activated by light or other energy source, for example, due to absorbance of energy or mechanical ultrasonic vibration thus achieving activation energy or causing instability resulting in destruction based on hyper-thermal damage.

Exothermic compositions may be biologically compatible explosives containing fuel and sufficient oxygen or oxidizer so that upon activation they are capable of undergoing a chemical change at a relatively high rate and speed, or at a speed approaching instant speed, resulting in the production of usable force through chemical change to produce heat and a gaseous product.

The exothermic compositions may also be compounds containing fuel and sufficient oxygen or oxidizers which are capable of releasing gases and heat, albeit at a lower rate than the explosives and includes such compositions or components thereof which react or are capable of reacting to yield usable quantities of heat with or without chemical products, including oxidatives. Examples of exothermic compositions are NaN_3 polysaccharide resins including organometallic or organo compounds, nitrocellulose and the like, nitrated metallorganic compounds, phosphorus compounds, compounds containing metal hydride with hydrocarbon and the like, inorganic nitrogen-oxygen salts including nitroglycerine, and the like. The activation of the exothermic compositions causes the destruction of the biological tissue by heat or by production of oxidatives, or production of usable mechanical force.

The helper agents specified in (i)-(iii) above should be mostly used for external applications, for example, where the light and photosensitizable compound, (in the photodynamic aspect) or the ultrasound, with a sonosensitizable compound, (in the sonodynamic aspect) are used to destroy biological tissue present on the external surface of a subject, such as on the skin, digestive tract, reproductive system, etc. The tissue may be epithelial cancerous or precancerous tissue, beauty spots, broken capillaries, birth marks, wrinkles, hair roots, etc.

The composition of matter capable of undergoing an exothermic reaction may be also used for destruction of internal tissues of the body, if it belongs to the type that releases heat at a relatively slow rate with relatively low

or no release of gaseous products, for example, said composition can be administered to a desired location inside the body, such as by injection, and then an ultrasound beam or a light beam carried by an optic fiber is administered to the location of injection. Destruction of an internal location inside a subject may be carried out in order to destroy a tumor, to block blood vessels, to open a blood clot and the like.

The methods according to the invention may also be used for enhancing the sterilizing effect of energy irradiation such as light, ultrasound, microwave, etc. when required to destroy undesired microorganisms such as bacteria, protozoa, parasites and the like, and in such a case it is possible for example to add to the solution in which the sterilization by the irradiation also one of the helper agents ((i) - (iii)) or the composition of matter capable of undergoing an exothermic reaction. In such a case the term *"biological tissue"* should be understood as referring also to single cell organisms.

It is also possible in accordance with the present invention to use energy irradiation such as ultrasound and light, optionally together with an agent capable of being sensitized by said irradiation such as photosensitizable/ sonosensitizable compounds and at least one of the helper agents (i) - (iii) or the composition of matter capable of undergoing an exothermic reaction in order to destroy biological tissue.

In accordance with a third aspect of the present invention, it has been found that an energy irradiation such as light, ultrasound or microwave irradiation causes precipitation, decomposition, and/or agglutination of photosensitizable or sonosensitizable compounds, respectively, thus terminating their destructive activity. Therefore, according to the method of the third aspect of the invention, after administering a photosensitizable or sonosensitizable agent to a subject, and activating it for a desired period of time by an energy irradiation, it is possible to terminate its destructive activity by application of energy, and thus, eliminate or significantly reduce the severe problem of prolonged light

sensitivity, which greatly hindered the widespread practice of photodynamic therapy (Orenstein *et al., supra*). The method of the third aspect of the invention thus enables to control the period in which the photosensitizable/sonosensitizable agent is active only to the period of the medical treatment.

Thus the present invention concerns a method for terminating the destructive activity of photosensitizable/sonosensitizable compounds, by application of an energy irradiation pulse capable of precipitating, or cause decomposition or agglutination of said photosensitizable compounds.

The parameters of the pulse (for example, light, ultrasound microwave) required for precipitation, decomposition and/or agglutination of the photosensitizable/sonosensitizable compound, may be determined empirically, for example, by direct measurement of the fluorescence activity of the sensitizer under different irradiation regimes, or by measuring the slope of effect evoked on final substract of the sensitizer activity, such a tryptophan, under different radiation regimes which change may be easily determined by a fluorescence or activity effect on substrate. The parameters of energy irradiation which are sufficient to terminate or significantly reduce said change in fluorescence can be used, in accordance with the fifth aspect of the invention.

For each photosensitizable/sonosensitizable compound, it is possible to empirically determine the exact parameters (frequency, duration, intensity) of the irradiation, necessary in order to terminate its destructive activity.

Typically, where the irradiation is ultrasound, the range of the ultrasound pulse required for the deactivation of a common photosensitizable compound such as, porphyrins, hematoporphyrins, chlorines, purpurins, phtalocyanines, photofrin, rhodamines, acridine derivated, rhodamines and the like or sonosensitizers (including e.g. gallium-porphyrin, DMSO (dimethylsulfoxide), DMF (dimethylformamide), mesoporphyrin, adramycin and mitomycin and the like are as follows:

Frequency: 20 KHz to 20 MHz, preferably 0.1 MHz to 10 MHz, most preferably 0.5 MHz to 5 MHz.

Intensity: 0.1 w/cm^2 to 500 w/cm^2, preferably 0.5 w/cm^2 to 100 w/cm^2, most preferably 2 w/cm^2 to 8 w/cm^2.

Duration: 0.5 sec. to 5 hrs, preferably 30 sec. to 1 hr, most preferably 2 min. to 40 min. under continuous wave mode. Extended periods may be required when using a pulsative mode.

It should be noted that there exists a reverse relationship between the intensity and the duration, i.e. the lower the intensity (above threshold of activation) the longer the duration should be. Therefore, for each specific photosensitizable compound there exists several pulses which can terminate its destructive activity, some of which have a relatively long duration of low intensity and some with short duration of high intensity.

Where the photosensitizable/sonosensitizable compound is administered to external surfaces of the body, it may be deactivated by application of unfocused wave of ultrasound. Where the photosensitizable compound is administered to deeper regions of the body, for example, by injection, perfusion or transdermal delivery as disclosed in co-pending Israel Application 119827 incorporated herein by reference, and is activated by light, for example, from an optic fiber or by ultrasound irradiation, the deactivation should be carried out by utilizing ultrasound capable of penetrating deep inside the body, for example, as disclosed in co-pending Israel Application 120079 incorporated herein by reference, or by unfocused ultrasound.

The photosensitizable or sonosensitizable agent may also be destructed by ultrasound irradiation having the resonance frequency of the particular sensitizer used. Such resonance frequency may also cause decomposition, agglutination or precipitation of the sensitizer. Generally, the state of the art sensitizer is in the range of 1 MHz to 10 GHg. By another aspect, it is possible to use irradiation of the resonance frequency for activation of

initially inactive photo- or sonosensitizers. The sensitizers are applied in an inactive form, for example, conjugated to an inactivating moiety. Irradiation of the resonance frequency caused cleavage of the active sensitizer from the inactivating moiety, thus causing its activation.

By a fourth aspect of the invention there is provided a method for terminating the destructive activity of photosensitizable and/or sonosensitizable compounds by activating exothermic compositions in the vicinity of said photosensitizable or sonosensitizable compounds thereby causing decomposition of the compounds.

In accordance with the fourth aspect of the invention, the photosensitizable or sonosensitizable compound is destroyed by compositions of matter capable of pertaining a exothermic reaction as explained above. The exothermic compositions should be administered before, together with, or after the photosensitizable or sonosensitizable compounds.

When the composition of matter capable of undergoing an exothermic reaction is delivered for cease of activity together with the actual sensitizer, then levels of energy irradiation such as light and/or ultrasound irradiation having such parameters which are capable of activating the photosensitizable compound without activating the composition of matter capable of undergoing an exothermic reaction should be used during treatment so as to cause the controlled destruction of biological tissue. At the end of treatment, when it is desired to terminate the destructive activity of the photosensitizable or sonosensitizable compound, an effective energy irradiation such as light or ultrasound irradiation level should be applied which is sufficient to activate the composition of matter capable of undergoing an exothermic reaction so as to produce sufficient heat, which can then decompose the photosensitizable or sonosensitizable agent and terminate or significantly reduce its destructive activity. The levels of irradiation should be empirically determined.

In the following, the invention will be disclosed with reference to some non limiting examples.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows reduction of fluorescence of $1x10^{-5}$ M Tryptophan in the presence of $2x10^{-6}$ M of the photosensitizable compound $TPPS_2$ after ultrasound irradiation of 3 MHz, continuous wave, 2.2 w/cm^2, for 20 mins, at 23°C;

Fig. 2 shows reduction of fluorescence of $1x10^{-5}$ M Tryptophan in the presence of $1x10^{-6}$ M of the photosensitizable compound $TPPS_2$ after ultrasound irradiation of 1 MHz, continuous wave, 1.7 w/cm^2, for 2 mins, at 23°C;

Fig. 3 shows reduction of fluorescence of $1x10^{-5}$ M Tryptophan in the presence of $2x10^{-6}$ M of the photosensitizable compound $TPPS_2$ after ultrasound irradiation of 1 MHz, continuous wave, 2.2 w/cm^2, for 2 mins, at 23°C; carried out after bubbling oxygen into the solution (for 60 secs.) thus elevating the oxygen concentration of the solute from 8 to 20 ppm;

Fig. 4 shows reduction of fluorescence of $1x10^{-5}$ M Tryptophan in the presence of $2x10^{-6}$ M of the photosensitizable compound $TPPS_2$ after ultrasound irradiation of 1 MHz, carried out after bubbling of oxygen as described above. The compounds were subjected to two ultrasound pulses of continuous wave the first for 2 min. at 1.7 w/cm^2 without oxygen bubbling and the second for another 1 min. at 2.2 w/cm^2 during bubbling of oxygen;

Fig. 5 shows reduction of fluorescence of $2x10^{-6}$ M of the photosensitizable compound $TPPS_2$ in the presence of $1x10^{-5}$ M Tryptophan after ultrasound irradiation of 1 MHz, continuous wave, 1.7 w/cm^2, for 30 mins, at 23°C;

Fig. 6 shows reduction of fluorescence of both $1x10^{-5}$ M Tryptophan and the photosensitizable $TPPS_4$ concentration of $5x10^{-7}$ M after ultrasound irradiation of 1 MHz, continuous wave, 1.7 w/cm^2, for 50 mins, at 23°C (a = absolute fluorescent activity of $TPPS_4$ and Tryptophan; b = relative fluorescent activity of each component);

Fig. 7 shows a schematic representation for a system for use in the method of the invention where the energy source is ultrasound; and

Fig. 8 shows reduction of fluorescent levels of tryptophan alone (10^{-5}M); tryptophan (10^{-5}M) and $TPPS_4$ (10^{-5}M); and $TPPS_4$ alone; after microwave irradiation of 2450 MHz, 34.4 W for 1 min.

DETAILED DESCRIPTION OF THE INVENTION

Example 1 Improved activation and deactivation of photo- and sonosensitizable compounds

A. Experimental set up

Three typical photosensitizable compounds, $TPPS_2$, $TPPS_4$ and $AlPcS_3$ at concentrations of 10^{-7}-10^{-5} M were used for ultrasound activation and deactivation. Qualitatively similar results were found when tryptophan was ultrasonically irradiated in the presence of each of the above-mentioned sensitizers. A Mettler sonicator 720 was used and the ultrasonic parameters were intensities of 1-2.2 W/cm^2 continuous wave; 1 or 3 MHz applied for different periods - up to 60 mins. Occasionally irradiation was carried out during or after bubbling of oxygen (O_2) into the solution and elevation of the oxygen content from 8 up to 25 ppm. When bubbling was performed, the solution was left for 1 minute before measuring fluorescence activity, to allow the escape of gas bubbles. Irradiation was always carried out in cooler bath with stirrers and the temperature was kept constant, mostly room temperature of 23°C. Irradiation was carried out in the presence of tryptophan at concentrations of 10^{-5}-10^{-6} M.

B. Results

It was found that ultrasound by itself had no effect on the tryptophan concentration, i.e. the fluorescence of tryptophan remained constant after irradiation. Tryptophan serves an acceptor for activated oxygen, when produced by the photosensitizable compounds. The oxygen affects mostly

double-bonds of the tryptophan thus reducing the concentration of the original form of tryptophan and this is revealed by decrease in the tryptophan fluorescence activity at the specific wave length. The remaining measured fluorescence is relevant to the concentration of tryptophan that remained in its original form, i.e. that was not changed by the sonosensitizable compounds. Similarly, fluorescence activity specific to the original forms of sensitizer was determined and the reduction of this activity after irradiation was measured.

In one example summarized in Fig. 1, $2x10^{-6}$ M of the photosensitizable agent $TPPS_2$ were ultrasound irradiated, in the presence of tryptophan concentration of 10^{-5} M, and irradiation parameters carried out at 3 MHz, intensity of 2.2 W/cm^2 continuous wave for 20 mins. at constant solution temperature of 23°C. The irradiation caused a significant decrease of about 35% in the concentrations of the original form of tryptophan. In a similar experiment using irradiation at 1 MHz, but otherwise under similar conditions, featured a 39% decrease (i.e. a 10% improvement as compared to the above experiment).

According to another example summarized in Fig. 2 utilizing ultrasound irradiation with continuous wave, intensity of 1.7 W/cm^2 at 1 MHz, for different periods up to 60 mins. 23°C was used. The sensitizer used was $TPPS_2$ at concentration of $1x10^{-6}$ M and tryptophan was at the concentration of $1x10^{-5}$. Measurements of fluorescence activity were carried out during 60 mins. The irradiation caused a significant and time dependent decrease of about 49% in the concentration of the original form of tryptophan.

According to yet another example summarized in Fig. 3 utilizing ultrasound irradiation at a continuous wave, intensity of 2.2 W/cm^2, 1 MHz, at 23°C - constant was used, the sensitizer being $TPPS_2$ at concentration of $2x10^{-6}$ M; in the presence of tryptophan at concentrations of $1x10^{-5}$. Irradiation was carried out after bubbling oxygen (O_2) in the solution for 60 seconds immediately before the irradiation, which resulted in an elevation of the oxygen concentration in the solution from 8 to 20 ppm, as was measured using Oxymeter (OxyGuard

Handy, MK II, Oxyguard, USA). Irradiation under these conditions, for only 2 mins. caused a 60% reduction of fluorescence activity of the tryptophan.

According to another example summarized in Fig. 4 a continuous wave of ultrasound irradiation 1 MHz, at 23°C constant was used, and the sensitizer $TPPS_2$ at concentration of $1x10^{-6}$M, and tryptophan at concentration of $1x10^{-5}$ were utilized. Irradiation was carried out after bubbling oxygen (O_2) in the solution to concentrations similar to those mentioned in relation to the previous example. The first irradiation was for 2 minutes at intensity of 1.7 W/cm^2 and was resulted in decrease of 43% of the tryptophan concentration. The same solution was re-irradiated for another 1 min. at intensity of 2.2 W/cm^2 during bubbling of oxygen. This was followed by another reduction of about 27% in the fluorescence activity of tryptophan and the total reduction during the 3 minutes of irradiation was of 70%. These results indicate that ultrasound activation is increased when supply of oxygen is provided, supporting the first aspect of the invention.

According to another example summarized in Fig. 5 continuous wave of ultrasound irradiation having the intensity of 1.7 W/cm^2 at 1 MHz was used, at a period up to 30 minutes, 23°C - constant, where the sensitizer was $TPPS_2$ at concentration of $1x10^{-6}$ M, and tryptophan concentrations were $1x10^{-5}$. This time the fluorescence activity of the sensitizer only was measured at different periods. The irradiation caused a 7% reduction of fluorescence activity of the sensitizer already after 10 mins. of irradiation and another 23% of reduction (total of 30%) after another period of 20 mins (30 mins. in total) of irradiation. The reduction of the concentration of the original form of the sensitizer was expected to cause a reduction in the slope (i.e. in the rate) of tryptophan response, as was found in Example 6, and is therefore directly associated to reduction in its effectiveness. The reduced activity of the sensitizer has the meaning of immediate reduction in the delayed sensitivity to light or ultrasound.

According to another example summarized in Fig. 6, continuous wave of ultrasound irradiation having intensity of 1.7 W/cm^2 at 1 MHz was used, for different periods of up to 50 mins. at 23°C constant, the sensitizer was $TPPS_4$ $5x10^{-7}$ M, and tryptophan concentration $1x10^{-5}$. Fluorescence activity of both tryptophan and $TPPS_4$ were measured every 10 mins. The irradiation caused a significant reduction of fluorescence activity of both the sensitizer and the tryptophan concentration (Fig. 6a) where initially the slope of reduction of tryptophan concentration is higher and later, when reduction of the concentration of the initial form of the sensitizer itself appears as reflected in the reduction of fluorescence from 10 mins., the slope or rate of reduction of tryptophan concentrating is markedly decreased. In terms of relations between the initial and the final concentrations (Fig. 6b) it becomes evident that the final concentration of the sensitizer after 50 mins. under the experimental regime, is only 28% of the initial concentration. This effect was carried out with destruction of 53% of the tryptophan with enhanced efficiency during the initial part of the irradiation period and reduced efficiency later, when the concentrations of the sensitizer itself reduced. 62% of the effect of destruction of tryptophan were achieved within the first 40% of the time, and later when the sensitizer was less available - the effect on tryptophan was also reduced. These results indicate that under the experimental irradiation the sensitizer activity was diminished, with remaining of only 28% of its original active concentration. It is equal to remaining of only 28% of the effect of delayed sensitivity, support the second aspect of the invention.

As can be seen, it is possible by utilizing various ultrasound parameters to reduce the activity of photosensitizable compounds. It is assumed that the reduction is partially due to precipitation, since if a detergent which opens aggregates and precipitates such as triton was used, there was partial increase in fluorescence of the sensitizer agent of about 30-50%. The remaining percent of fluorescence, i.e. 50-70% which was not restored by use of a detergent indicates

that other, unknown mechanisms contribute to the deactivation of photosensitizable compounds by ultrasound.

Example 2 A system for administration of ultrasound

Fig. 7 shows a system for carrying out the method of the invention, for destruction of biological tissue by ultrasound irradiation. The system **1** is composed of a treatment container device **2**; an energy irradiation radiation source **3** (being in the present case an ultrasonic energy source); an energy delivery member **4**, being in the present case an ultrasonic transducer; a first reservoir **5** containing the medicament or sensitizable agent; a second reservoir **6** containing the helper agents; and a computerized control unit **7**.

Treatment container device **2**, which hold the active agents preferably made of plexyglass and sensitized by the irradiation source (for example sonosensitizable agents) is in the form of a container In practice, the opening **8** of container **2** is placed on the tissue to be treated, for example a skin region of the patient which is to be destroyed due to malignancy.

The sonosensitizable agents within the container, are activated by ultrasonic irradiation originating from source **3**, comprising a signal generator, an amplifier and optionally a matching unit. The ultrasonic energy is then transferred to energy delivery member **4** which in the present case is an ultrasonic transducer. The container **2** is attached to a member **4** through irradiation area **9** which preferably is in the form of a plate. Ultrasonic irradiation delivery member **4** is composed of at least one transducer capable of producing either a regular or focused beam of different frequencies, intensities, pulse modes and duration, for the activation and/or decomposition of said sonosensitizable agent.

The distance between the energy delivery member **4** and opening **8** is adjustable, by use of threadings **10** and motor **11**, which can change the overall length of container **2**. Said change alters the position of the ultrasonic energy in relation with opening **8**, and thus determine the place or depth in which the focus

of the ultrasonic beam will be created, or the distribution of the ultrasound energy.

Container **2** has two openings therein: outlet **12** and inlet **13**. Solution is circulated between the two openings, through filter **16** and reservoir **5**, by the aid of pump **14**. Filter **16** is intended to purify the circulating solution from various impurities which may be due to release of various components from the treated biological tissue (for example, skin cells), or agglutinates of the sonosensitizable compositions or helper agents. Outlet **12** and inlet **13** can each be independently closed according to the desired affect. According to one application, inlet **13** is closed while pump **14** is active resulting in a suction affect of the solution and cellular debris from opening **8** attached to the target tissue, and thus via tube **15** through filter **16**, the solution reaches the reservoir **5**.

The level of the active components which are present in the solution recycled into reservoir **5**, is detected using probes **17** and **21**. Said probes are suitable to detect the level of the sonosensitizable helper agent, (or any other additive agent) so that the solution may be replenished, in order to maintain a predefined level of helper or additive. For example, the probe may be an oxygen electrode. If the level of the helper agent (i.e. O_2) is too low as detected by the probes, additional O_2 rich agents can be pumped by pump **18** from second reservoir **6** into first reservoir **5**, and from there to the container **2**. In reservoir **5**, the added solution is mixed with the recycled solution by stirrer **19**.

If it is desired, samples of the solutions present in reservoir **5** can be obtained to an external container by tap **20** for various monitoring and chemical determination. The whole system is controlled by computerized control unit **7** which measures and collects information from detectors **17** and **21**, measures the level and volume of the components in reservoirs **5** and **6** and in treatment container device **2**.

For example, when the helper agent is gas such as O_2, the system can detect the appearance of undesired bubbles in the epical part of container **2**

close to opening **8**. Computerized control unit **7** controls the activity of pumps **14** and **18** and of motor **11**, which determines the distance between unit **4** and opening **8**. The computerized system also controls the operation of irradiation source **3** coupled to transducer **4**, and determines the intensity, frequency and duration of the pulses given.

The system as shown in the figure, is applicable for external tissues such as epithelial tissues, as well as moist epithelial tissues of the digestive tract, reproductive system, eye epithelium, and the like. However, this system may also be used for internal purposes, wherein the container treatment device **2** is minimized and constructed in a manner suitable for internal applications.

Example 3 Decomposition of sensitizable agents by microwave energy

An experimental set up similar to the one mentioned in Example 1 (A) was constructed. Irradiation was carried out using Amana RS520I microwave, 2450 MHz, 34.4 W for 1 min. Irradiation was carried out on the following solutions at time t = 0 and for 1 min.:

Solution (a) – tryptophan (10^{-5}M) alone (green and brown peaks – for t = 0 and 1 respectively) peaks around 280 nm.

Solution (b) – tryptophan (10^{-5}M) and $TPPS_4$ (10^{-6}M) (red and yellow for t = 0 and 1 respectively) peaks around 280 nm (tryptophan) and 420 nm ($TPPS_4$).

Solution (c) – $TPPS_4$ (10^{-6}M) alone (blue and purple for t = 0 and 1 min. respectively).

The results are shown in Fig. 8 wherein: 1 is the results for $TPPS_4$ alone at t = 0; 2 is the results for $TPPS_4$ when present with tryptophan at t = 0; 3 is the results for $TPPS_4$ alone at t = 1 min.; 4 is the results for $TPPS_4$ when present with tryptophan at t = 1 min.; 5,6 are the results for tryptophan alone at t = 0 and t = 1 min.

For each sample the area below the peak (~ 420 nm) was measured as an indicator of the amount of the original form of the sensitizer. For each of the 3 solutions the area between 375 and 435 nm was measured above the tangential line at these points and was compared to the original area (at t = 0 of the appropriate control).

Data show that while tryptophan remained constant (i.e. non-altered), alterations appeared in the area (which is an indicator of the concentration of the original form) of the sensitizer $TPPS_4$. In solution (a): since there was no sensitizer there was no alternation. In solution (b): the area reduced from 54.1 to 49.5 – a reduction of about 9% in the concentration of the original form of the sensitizer. In solution (c): the area reduced from 52.9 to 49.3 – a reduction of about 7% in the concentration of the original form of the sensitizer. The results indicate that decomposing of the active component of the solution can be performed using irradiation.

CLAIMS:

1. A method for the destruction of biological tissue by an energy irradiation comprising exposing the biological tissue to energy irradiation in the presence of an agent capable of being sensitized by said irradiation, and at least one agent selected from the group consisting of:

(i) a compound capable of releasing singlet oxygen;

(ii) a compound capable of releasing oxidating agents; and

(iii) a compound capable of releasing free radicals.

2. A method for the destruction of tissue by energy irradiation comprising: exposing the biological tissue to irradiation in the presence of a composition of matter which upon exposure to said irradiation undergoes an exothermic reaction.

3. A method according to Claim 1, for the destruction of biological tissue by ultrasound irradiation comprising: exposing the biological tissue to ultrasound irradiation in the presence of a sonosensitizable agent and at least one agent selected from the group consisting of:

(i) a compound capable of releasing singlet oxygen;

(ii) a compound capable of releasing oxidating agents; and

(iii) a compound capable of releasing free radicals.

4. A method according to Claim 2, for the destruction of biological tissue by ultrasound irradiation comprising: exposing the biological tissue to ultrasound irradiation in the presence of a composition of matter which, upon exposure to said irradiation undergoes an exothermic reaction.

5. A method according to Claim 4, wherein the ultrasound irradiation is carried out further in the presence of at least one agent selected from the group consisting of:

(i) a compound capable of releasing singlet oxygen;

(ii) a compound capable of releasing oxidating agents; and

(iii) a compound capable of releasing free radicals.

6. A method according to Claim 4, wherein the ultrasound irradiation is carried out further in the presence of at least one other sonosensitizable agent.

7. A method according to Claim 3 or 6, wherein the sonosensitizable agent is selected from the group consisting of gallium-porphyrin, DMSO (dimethylsulfoxide), DMF (dimethylformamide), mesoporphyrin, adramycin, mitomycin, porphyrins, hematoporphyrins, chlorines, purpurins, phthalocyanines, photofrin, rhodamines, acridine derivated and rhodamines.

8. A method according to Claim 1, for the destruction of biological tissue by light, comprising exposing the biological tissue to light in the presence of a photosensitizable agent and at least one agent selected from the group consisting of:

(i) a compound capable of releasing singlet oxygen;

(ii) a compound capable of releasing oxidating agents; and

(iii) a compound capable of releasing free radicals.

9. A method according to Claim 2, for the destruction of biological tissue by light comprising: exposing the biological tissue to light in the presence of a composition of matter which, upon exposure to said light, undergoes an exothermic reaction.

10. A method according to Claim 9, wherein the exposure to light is carried out further in the presence of an agent selected from the group consisting of:

(i) a compound capable of releasing singlet oxygen;

(ii) a compound capable of releasing oxidating agents; and

(iii) a compound capable of releasing free radicals.

11. A method according to Claim 9, wherein the exposure to light is carried out further in the presence of at least one other photosensitizable agent.

12. A method according to Claim 8 or 11, wherein the photosensitizable agent is selected from the group consisting of porphyrins,

hematoporphyrins, chlorines, purpurins, phthalocyanines, photofrin, rhodamines, acridine derivated and rhodamines.

13. A method according to any one of Claims 1, 3, 5, 8 or 10, wherein the compound capable of releasing singlet oxygen is selected from the group consisting of: O_2, H_2O_2, and kalium permanganat.

14. A method according to any one of Claims 1, 3, 5, 8 or 10, wherein the compound capable of releasing oxidative agents is selected from the group consisting of chloride, bromide, fluoride, iodine, alkali metal elements, alkali earth metals, oxygen and sulfur.

15. A method according to any one of Claims 1, 3, 5, 8 or 10, wherein the compound capable of releasing free radicals is selected from the group consisting of: N,N-dimethylbromide (DMF), N-Me formamide (NMF) and dimethyl superoxide (DMSO).

16. A method according to Claim 1 or 2, wherein the biological tissue is present on the external surface of a subject.

17. A method for the destruction of biological tissue present within a body of an individual, by an ultrasound beam, comprising: administering to the site of desired destruction a composition of matter which, upon exposure to ultrasound irradiation, undergoes an exothermic reaction, and then irradiating the site of desired destruction with a focused ultrasound irradiation capable of activating said composition of matter.

18. A method according to Claim 2, 4 or 9, wherein the composition of matter comprises a compound selected from the group consisting of: compositions having organic compounds containing nitrated metal; phosphorous compounds; inorganic metal fulminate or metal azide or free metal; hydrazine or hydrazine derivate; inorganic nitrogen - oxygen salts; nitrogen oxide or acid thereof; inorganic oxygen-halogen salt; forms of metal with hydrocarbon or halogenated, nitrated organic compounds, compounds containing free carbon; or compounds containing boron.

19. An agent for the destruction of tissue by energy irradiation therapy in conjunction with an agent capable of being sensitized by said energy irradiation said agent selected from the group consisting of:

(i) a compound capable of releasing singlet oxygen;

(ii) a compound capable of releasing oxidating agents; and

(iii) a compound capable of releasing free radicals.

20. An agent according to Claim 19, for the destruction of biological tissue by sonodynamic therapy in conjunction with sonosensitizable agent said agent selected from the group consisting of:

(i) a compound capable of releasing singlet oxygen;

(ii) a compound capable of releasing oxidating agents; and

(iii) a compound capable of releasing free radicals.

21. An agent according to Claim 19, for the destruction of biological tissue by photodynamic therapy in conjunction with photosensitizable compounds said agent selected from the group consisting of:

(i) a compound capable of releasing singlet oxygen;

(ii) a compound capable of releasing oxidating agents; and

(iii) a compound capable of releasing free radicals.

22. An agent according to Claim 19, 20 or 21, wherein the compound capable of releasing singlet oxygen is selected from the group consisting of: O_2, H_2O_2, and kalium permanganat.

23. An agent according to Claim 19, 20 or 21, wherein the compound capable of releasing oxidative agents is selected from the group consisting of chloride, bromide, fluoride, iodine, alkali metal elements, alkali earth metals, oxygen and sulfur.

24. An agent according to Claim 19, 20 or 21, wherein the compound capable of releasing free radicals is selected from the group consisting of: N,N-dimethylbromide (DMF), N-Me formamide (NMF) and dimethyl superoxide (DMSO).

25. An agent for the destruction of biological tissue by energy irradiation therapy being a composition of matter which undergoes an exothermic reaction upon said energy irradiation.

26. An agent according to Claim 25, for the destruction of biological tissue by sonodynamic therapy being a composition of matter which undergoes an exothermic reaction upon ultrasound irradiation.

27. An agent according to Claim 25, for the destruction of biological tissue by photodynamic therapy being a composition of matter which undergoes an exothermic reaction upon exposure to light.

28. An agent according to Claim 25 to 27, wherein the composition of matter comprises a compound selected from the group consisting of: compositions having organic compounds containing nitrated metal; phosphorous compounds; inorganic metal fulminate or metal azide or free metal; hydrazine or hydrazine derivate; inorganic nitrogen - oxygen salts; nitrogen oxide or acid thereof; inorganic oxygen-halogen salt; forms of metal with hydrocarbon or halogenated, nitrated organic compounds, compounds containing free carbon; or compounds containing boron.

29. A method for terminating the destructive activity of photosensitizable or sonosensitizable agents comprising: applying to the site of the photosensitizable/sonosensitizable agent an energy irradiation capable of causing decomposition, agglutination or precipitation of said agents.

30. A method according to Claim 29, for terminating the destructive activity of photosensitizable or sonosensitizable agents comprising: applying to the site of the photosensitizable/sonosensitizable agent ultrasound irradiation capable of causing decomposition, agglutination or precipitation of said agents.

31. A method according to Claim 30, wherein the ultrasound irradiation has the frequency of 20 KHz to 20 MHz, intensity of 0.1 to 500 W/cm^2 and duration of 0.5 sec. to 5 hours.

32. A method according to Claim 31, wherein the ultrasound irradiation has the frequency of 0.1 MHz to 10 MHz, intensity of 0.5 to 100 W/cm^2 and duration of 30 sec. to 1 hour.

33. A method according to Claim 32, wherein the ultrasound irradiation has the frequency of 0.5 MHz to 5 MHz, intensity of 2-8 W/cm^2 and duration of 2 min. to 40 min.

34. A method for the destruction of biological tissue at a desired site by light irradiation comprising:

(a) applying to the desired site a photosensitizable agent capable of being activated by a first light irradiation;

(b) activating said photosensitizable agent by said first light irradiation thereby causing destruction of the biological tissue at said site;

(c) applying to said site a composition of matter, which undergoes an exothermic reaction upon exposure to ultrasound or to a second light irradiation, thereby creating heat sufficient to cause decomposition, agglutination or precipitation of said photosensitizable agent, said second light irradiation having a higher intensity and/or duration than said first light irradiation;

(d) applying said ultrasound irradiation or said second light irradiation to said site.

35. A method for the destruction of biological tissue at a desired site by ultrasound irradiation comprising:

(a) applying to the desired site a sonosensitizable agent capable of being activated by a first ultrasound irradiation;

(b) activating said sonosensitizable agent by said first ultrasound irradiation thereby causing destruction of the biological tissue at said site;

(c) applying to said site a composition of matter which undergoes an exothermic reaction upon exposure to a second ultrasound irradiation thereby creating heat sufficient to decompose said sonosensitizable agent, said second

ultrasound irradiation having a higher intensity and/or duration and/or frequency than said first ultrasound irradiation;

(d) applying said second ultrasound irradiation to said site.

36. A method according to Claim 34, wherein said composition of matter is administered to said site together with the photosensitizable agent.

37. A method according to Claim 34, wherein said composition of matter is administered to said site after the administration of the photosensitizable agent.

38. A method according to Claim 35, wherein said composition of matter is administered to said site together with the sonosensitizable agent.

39. A method according to Claim 35, wherein said composition of matter is administered to said site after the administration of said sonosensitizable agent.

40. A system for use in any one of the methods of Claims 1 to 18 substantially as hereinbefore describe.

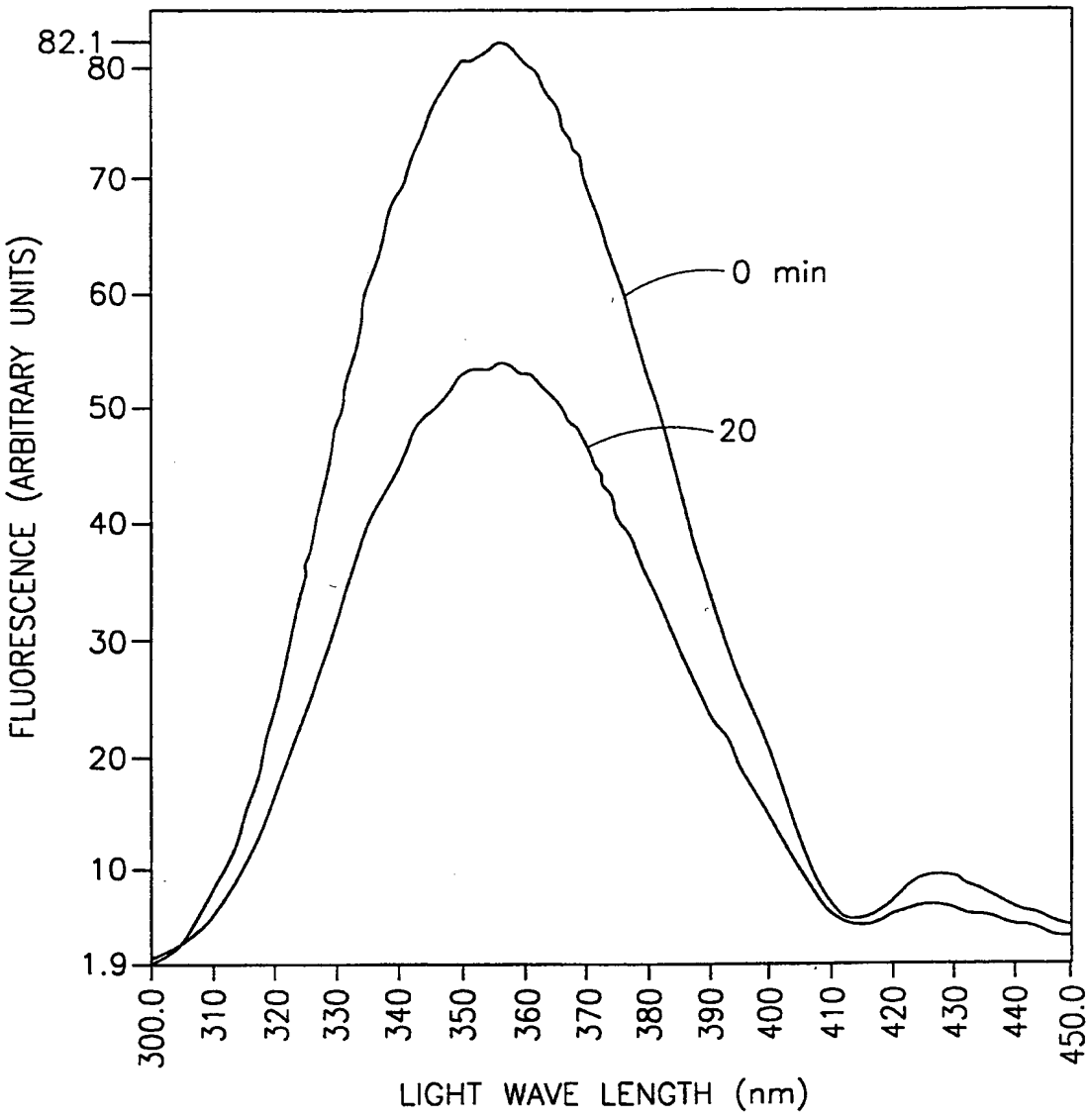
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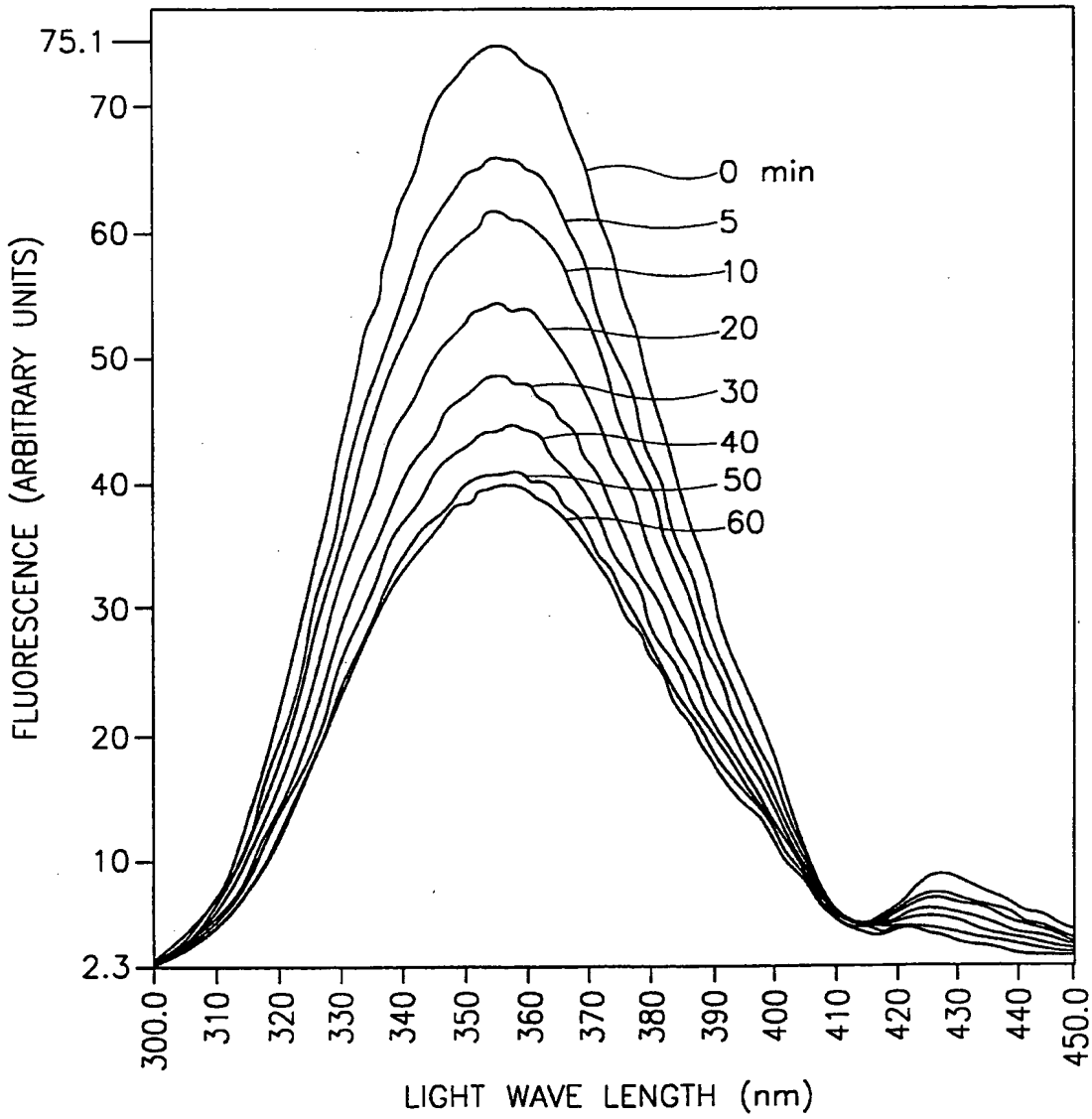
FIG.1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LIGHT WAVE LENGTH (nm)

FIG.2

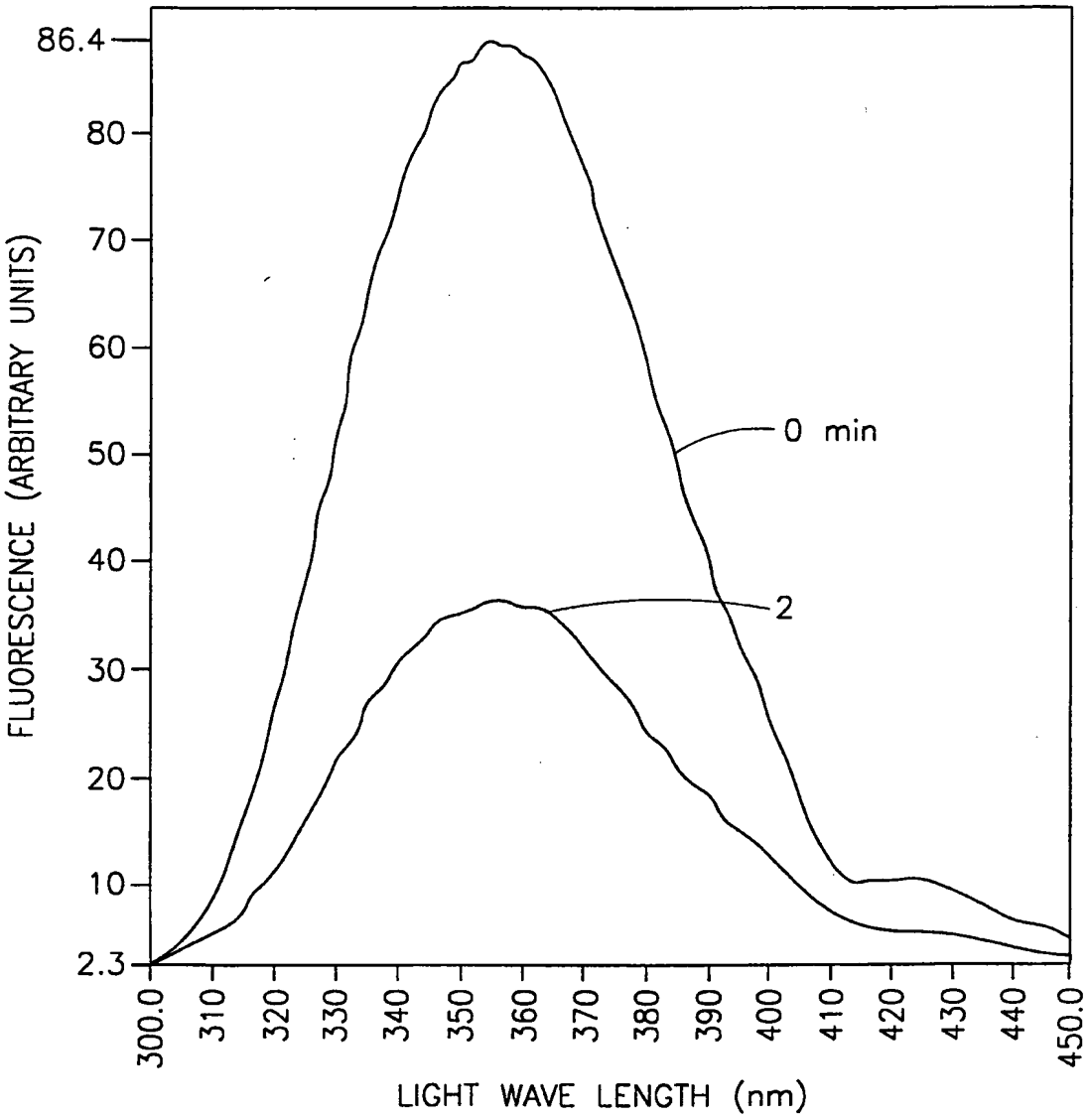
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FIG.3

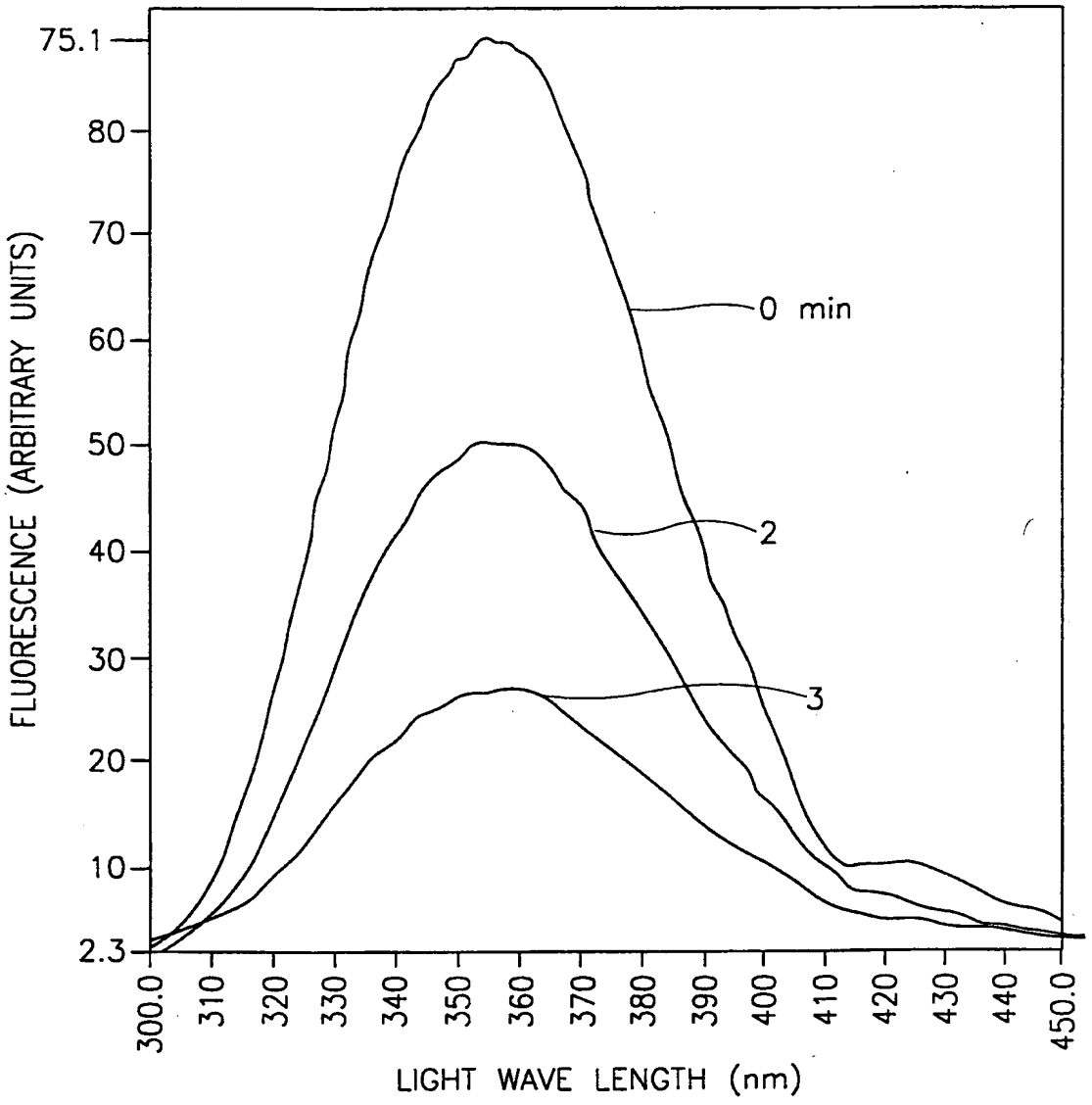
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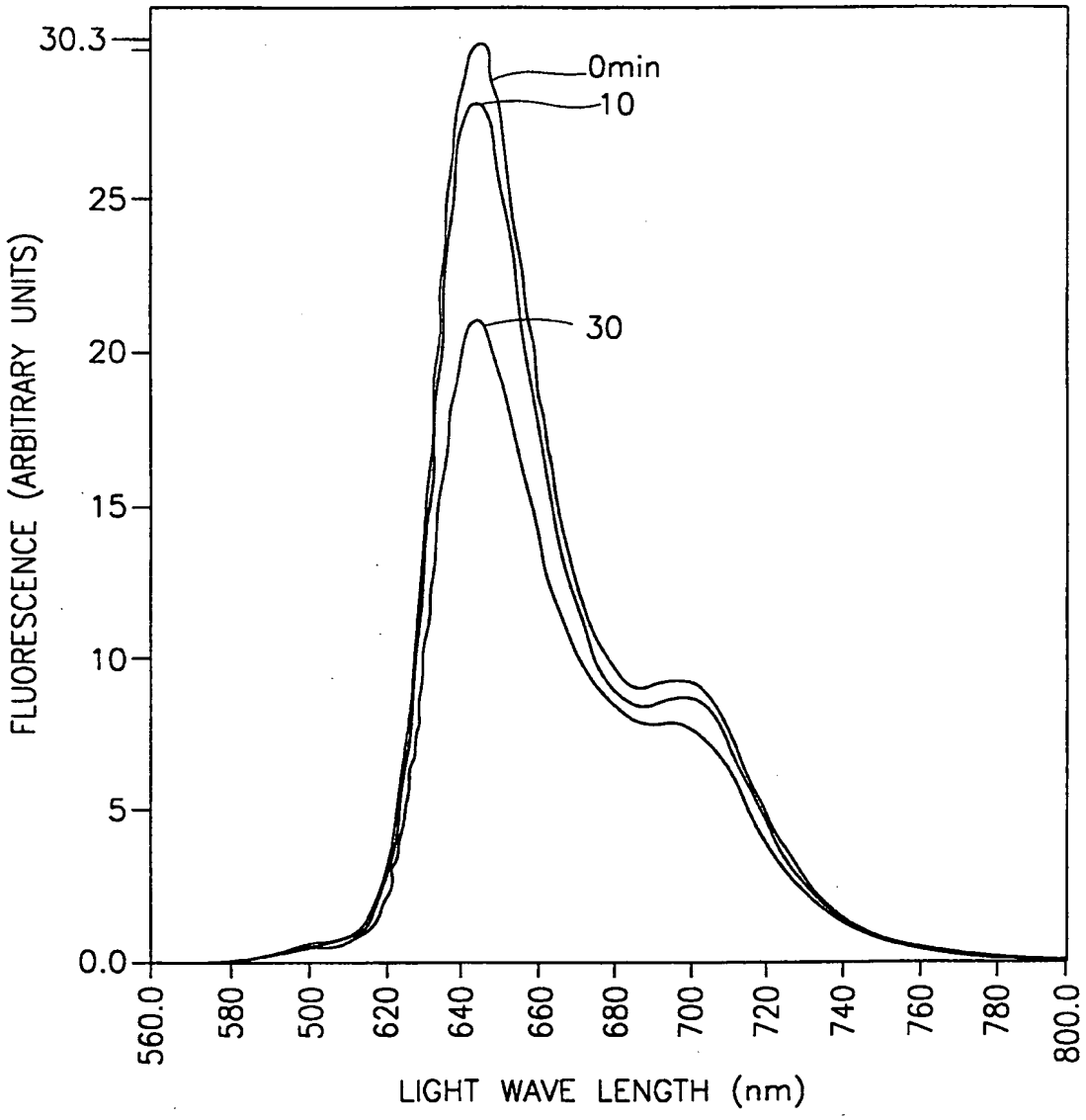
FIG.4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LIGHT WAVE LENGTH (nm)

FIG.5

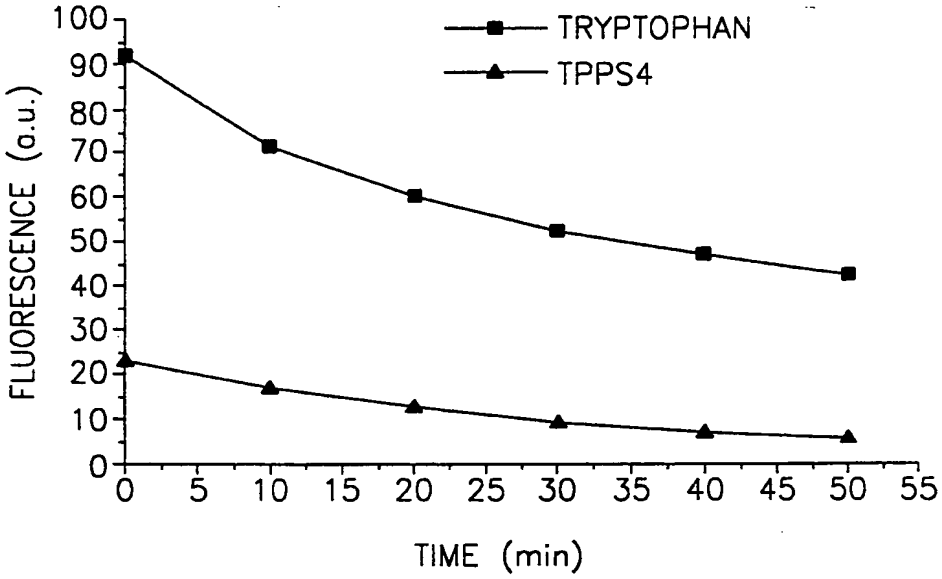
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FIG.6A

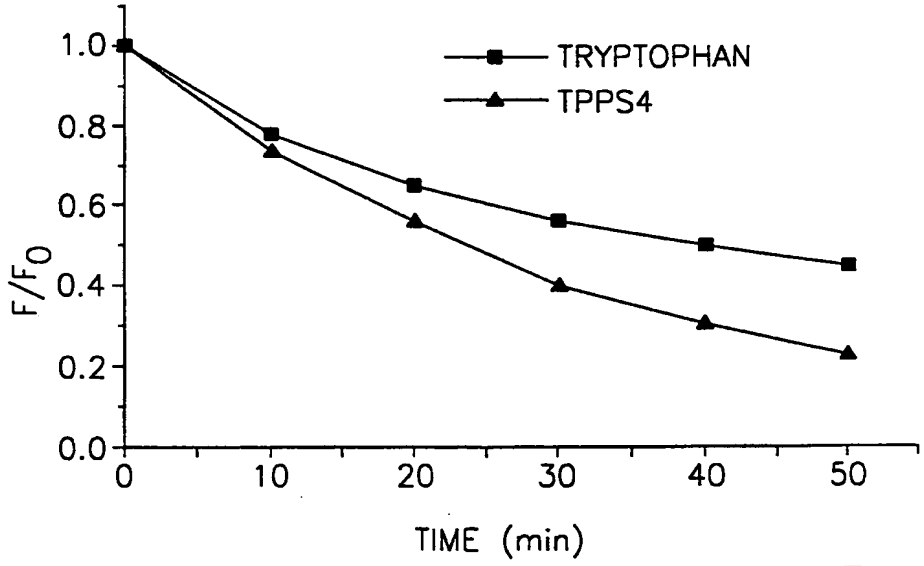
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FIG.6B

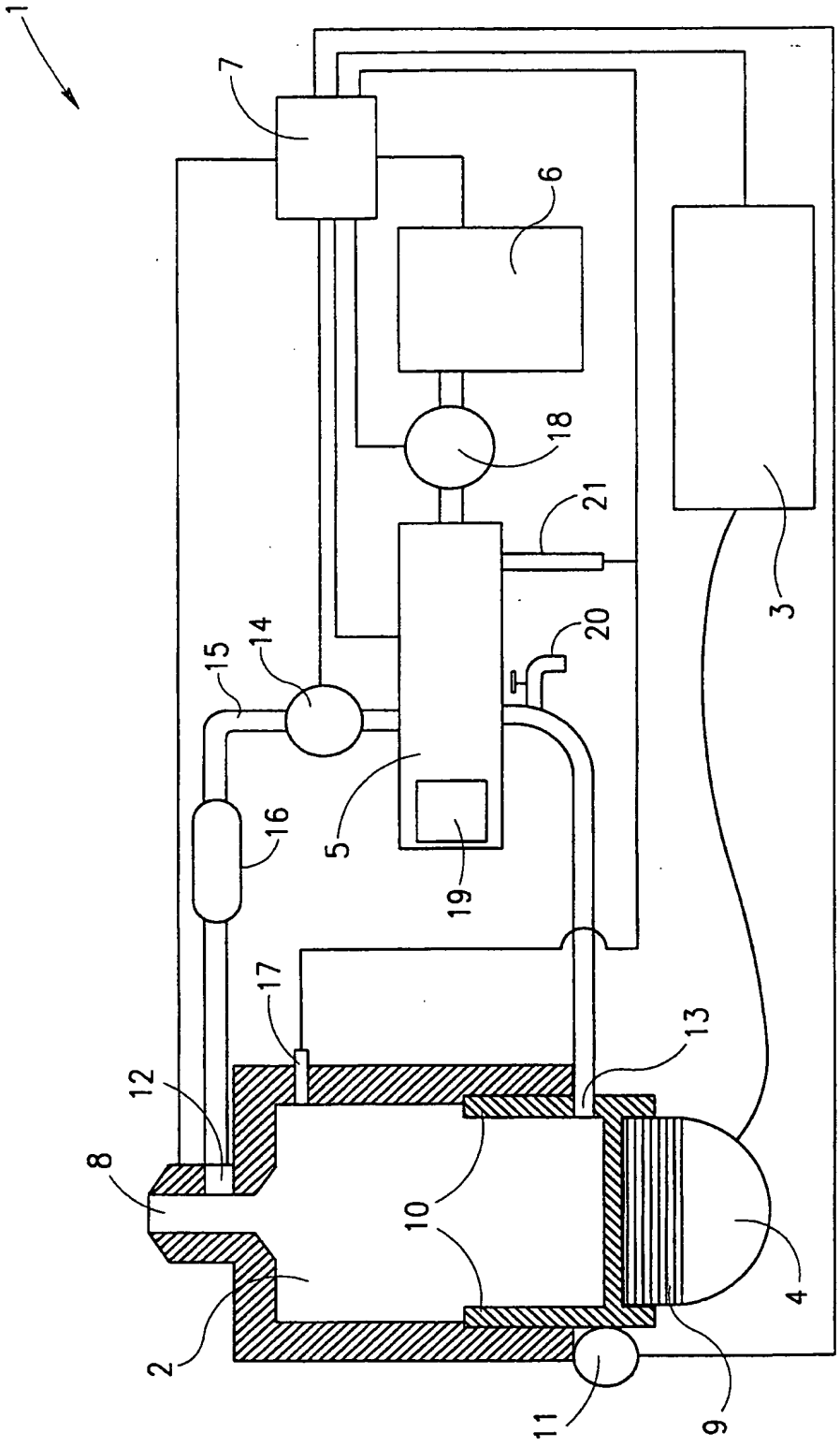
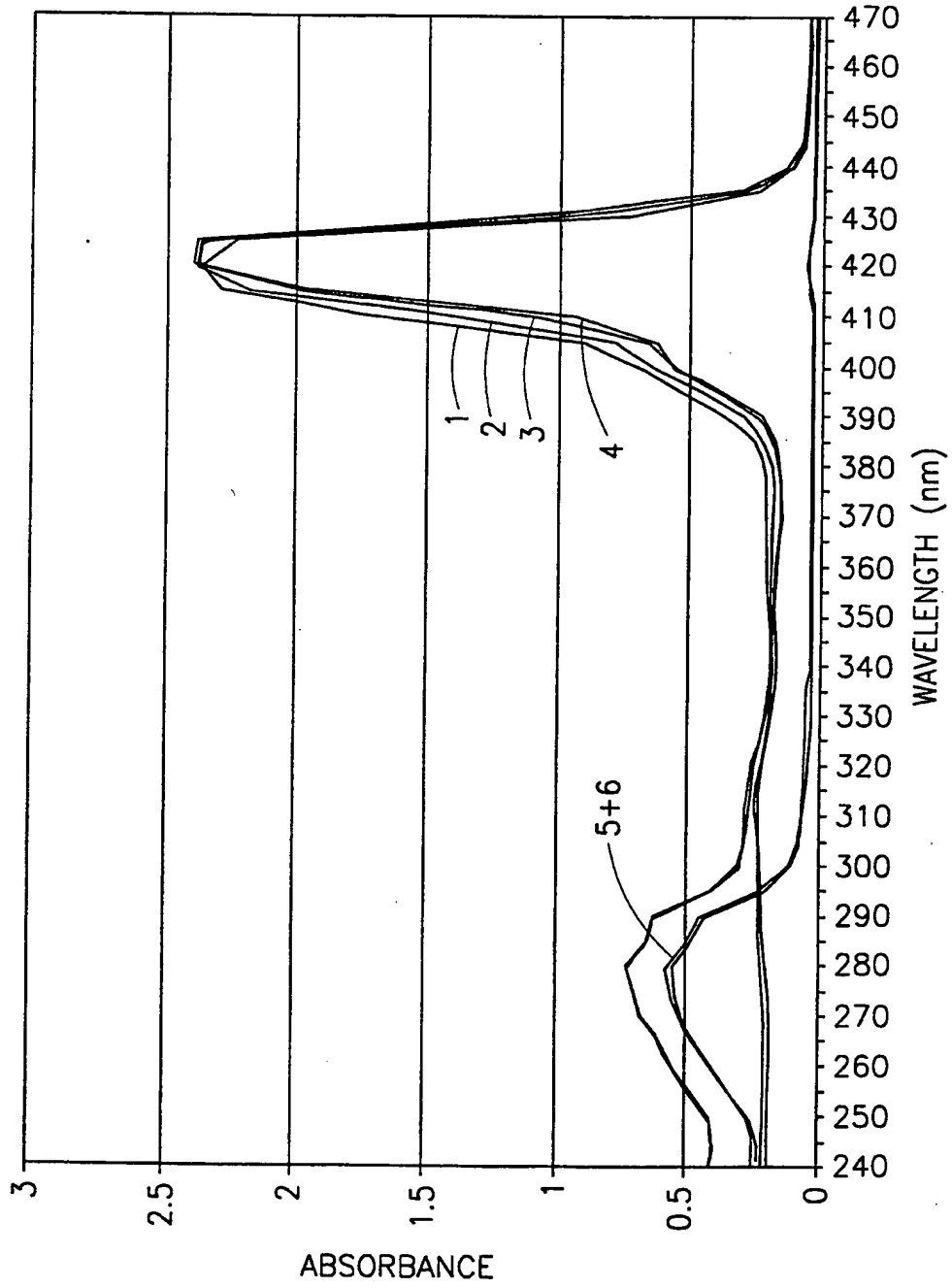


FIG.7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WAVELENGTH (nm)
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2
3
4
5+6
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2.5
2
1.5
1
0.5
0
ABSORBANCE

FIG.8

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IL 98/00231

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K41/00

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 98 01131 A (HITACHI LTD ;SUGITA NAMI (JP); SASAKI KAZUAKI (JP); KAWABATA KENIC) 15 January 1998 see claims ---	1-40
X	MIYOSHI, N. ET AL: "Effect of gallium-porphyrin analog ATX-70 on nitroxide formation from a cyclic secondary amine by ultrasound: On the mechanism of sonodynamic activation" PHOTOMED. PHOTOBIOL. (1995), 17, 139-40 CODEN: PHPHEA;ISSN: 0912-232X, XP002077399 see the whole document --- -/--	1-40

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 September 1998	28/09/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Berte, M

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category '	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KESSEL D ET AL: "Modes of photodynamic vs. sonodynamic cytotoxicity." JOURNAL OF PHOTOCHEMISTRY AND PHOTOBIOLOGY. B, BIOLOGY, (1995 JUN) 28 (3) 219-21. JOURNAL CODE: JLI. ISSN: 1011-1344., XP002077400 Switzerland cited in the application see figures	1-40
X	SUZUKI, TOSHIO ET AL: "A study on sonodynamic therapy-antitumor effect of novel sonodynamic compounds under ultrasound" HETEROCYCLES (1994), 38(6), 1209-11 CODEN: HTCYAM;ISSN: 0385-5414, XP002077401 see figure 2	1-40
P,X	MIYOSHI N ET AL: "Sonodynamic toxicity of gallium-porphyrin analogue ATX-70 in human leukemia cells." RADIATION RESEARCH, (1997 JUL) 148 (1) 43-7. JOURNAL CODE: QMP. ISSN: 0033-7587., XP002077402 United States see page 47, column 1, paragraph 2; figure 1 see page 44, column 2, paragraph 2	1-40
X	SHIN-ICHIRO UMEMURA ET AL: "SONOCHEMICAL ACTIVATION OF HEMATOPORPHYRIN: A POTENTIAL MODALITY FOR CANCER TREATMENT" PROCEEDINGS OF THE ULTRASONICS SYMPOSIUM, MONTREAL, OCT. 3 - 6, 1989, vol. VOL. 2, no. -, 3 October 1989, pages 955-960, XP000139558 MCAVOY B R see figures	1-40
X	JP 01 146829 A (KOSHIRO UMEMURA) 8 June 1989 see abstract	1-40

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 124, no. 9, 26 February 1996 Columbus, Ohio, US; abstract no. 105881, MISIK, VLADIMIR ET AL: "Peroxyl radical formation in aqueous solutions of N,N-dimethylformamide, N-methylformamide, and dimethylsulfoxide by ultrasound: implications for sonosensitized cell killing" XP002077404 cited in the application see abstract & FREE RADICAL BIOL. MED. (1996), 20(1), 129-38 CODEN: FRBMEH;ISSN: 0891-5849,1996, ---	1-40
X	R.J. JEFFERS ET AL.: "Dimethylformamide as an enhancer of cavitation-induced cell lysis in vitro." J. ACOUSTICAL SOC. AMER., vol. 97, no. 1-6, 1995, pages 669-676, XP002077403 cited in the application see abstract; figure 3 ---	1-40
X	CHEMICAL ABSTRACTS, vol. 123, no. 12, 18 September 1995 Columbus, Ohio, US; abstract no. 152697, MALIK, Z. ET AL: "Topical application of 5-aminolevulinic acid, DMSO and EDTA: protoporphyrin IX accumulation in skin and tumors of mice" XP002077405 see abstract & J. PHOTOCHEM. PHOTOBIOL., B (1995), 28(3), 213-18 CODEN: JPPBEG;ISSN: 1011-1344,1995, ---	1-40
X	CHEMICAL ABSTRACTS, vol. 125, no. 15, 7 October 1996 Columbus, Ohio, US; abstract no. 185070, MISIK, VLADIMIR ET AL: "EPR spin trapping study of the decomposition of azo compounds in aqueous solutions by ultrasound: potential for use as sonodynamic sensitizers for cell killing" XP002077406 see abstract & FREE RADICAL RES. (1996), 25(1), 13-22 CODEN: FRARER;ISSN: 1071-5762, -----	1-40

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL 98/00231

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim(s) 1-18, 29-39 is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
See FURTHER INFORMATION sheet PCT/ISAY/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/ IL 98/00231

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

In view of the large number of compounds, which are defined by the general definition in the independent claims, the search was limited to the compounds for which pharmacological data was given and/or the compounds mentioned in the claims, and to the general idea underlying the application. (See Guidelines, Chapter III, paragraph 2.3) Claims searched incompletely 1-40.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/IL 98/00231

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9801131 A	15-01-1998	NONE	
JP 01146829 A	08-06-1989	JP 1904371 C	08-02-1995
		JP 6029196 B	20-04-1994
		US 4971991 A	20-11-1990